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Comparison of Roche MONITOR and Organon Teknika NucliSens Assays To Quantify Human Immunodeficiency Virus Type 1 RNA in Cerebrospinal Fluid

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We compared Roche MONITOR and Organon Teknika NucliSens assays for human immunodeficiency virus type 1 (HIV-1) RNA in cerebrospinal fluid (CSF). Results of 282 assays were highly correlated ($r = 0.826$), with MONITOR values being 0.29 ± 0.4 $\log_{10}$ copies/ml (mean ± standard deviation) values. Both assays can reliably quantify HIV-1 RNA in CSF.

Human immunodeficiency virus type 1 (HIV-1) enters the central nervous system (CNS) during primary HIV infection and remains in the CNS throughout disease (5, 16). HIV-1 RNA concentrations in cerebrospinal fluid (CSF) correlate with cognitive abnormalities during AIDS (6, 9). Although antiretroviral therapy can control HIV-1 replication in peripheral tissues, the ability to control HIV-1 in the CNS is less well established, and there is evidence that some drugs do not effectively cross the blood-brain barrier (13). Both patient care and research related to HIV-1 infection of the CNS may benefit from validated methods to quantify virus in CSF.

We compared the performances of two commercially available HIV-1 RNA assays for quantitation of HIV-1 RNA in CSF: AMPLICOR HIV-1 MONITOR (Roche Diagnostic Systems, Branchburg, N.J.), and NucliSens HIV-1 QT (Organon Teknika, Durham, N.C.). The Roche MONITOR assay employs reverse transcriptase PCR technology to quantitate HIV-1 RNA. The Organon-Teknika NucliSens assay uses isothermal amplification of RNA through a process termed nucleic acid sequence-based amplification (14). Previous studies that compared the NucliSens assay and MONITOR assay using plasma samples demonstrated comparable sensitivities and linearities over most of the dynamic range, although the MONITOR assay performed somewhat better at lower HIV-1 RNA concentrations and NucliSens performed somewhat better at higher HIV-1 RNA concentrations (1–4, 8, 10, 12). The NucliSens assay extracts HIV-1 RNA onto silica beads, which may facilitate quantitation in some body fluids by removing PCR inhibitors (7). A recent evaluation which failed to detect inhibitors in CSF suggested that MONITOR and NucliSens assays may be comparable for this fluid (15). The present study compared to the NucliSens and MONITOR HIV-1 RNA as-

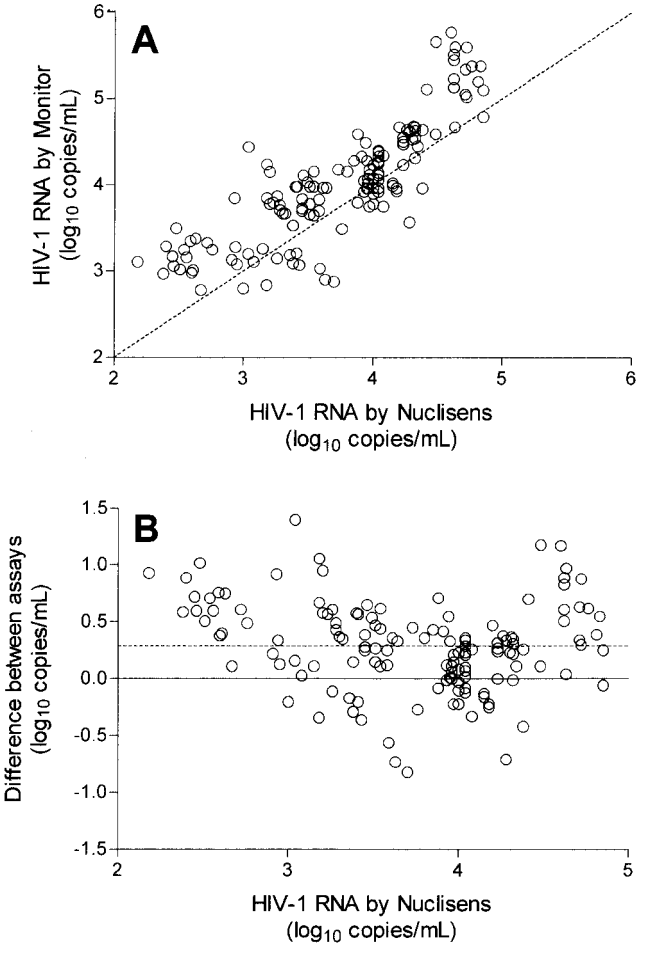

FIG. 1. Comparison of CSF HIV-1 RNA assay results by NucliSens assay versus MONITOR assay. (A) Scatter plot of data. The dashed line indicates the line of unity. (B) Scatter plot of difference between assay results (MONITOR assay value − NucliSens assay value). The dashed line indicates the mean difference between assay results.

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TABLE 1. Determinations of HIV-1 RNA in CSF by MONITOR assay and NucliSens assay at baseline

Patient no.	No. of samples	NucliSens assay		MONITOR assay	
		Mean $\log_{10}$ RNA	Variance	Mean $\log_{10}$ RNA	Variance
1	17	3.345	0.034	3.959	0.045
2	17	4.681	0.015	5.313	0.071
3	17	4.317	0.011	4.551	0.017
4	17	4.061	0.012	4.102	0.025
5	5	4.072	0.024	3.773	0.023

says in a large number of CSF samples obtained from patients before and after initiating antiretroviral therapy.

Subjects were HIV positive, antiretroviral naïve, and at least 18 years of age and had pretreatment HIV-1 RNA concentrations in CSF and plasma greater than 2,500 and 25,000 copies/ml, respectively (by NucliSens assay). Ultraintensive sampling of CSF via indwelling lumbar catheters was performed as described elsewhere (11). Briefly, lumbar CSF was sampled continuously for 48 h at baseline. Four days after catheter removal, therapy was initiated with stavudine, lamivudine, and nelfinavir. Beginning on treatment day 4, ultraintensive CSF sampling was again performed for 48 h. A total of 17 baseline and 17 on-treatment CSF samples from each subject, representing samples collected at 3-h intervals, were analyzed by both NucliSens and MONITOR assays. Five additional baseline samples were collected in an identical manner from a fifth subject. A total of 141 CSF samples were tested by each assay. The study was approved by the institution's Committee for the Protection of Human Subjects, and all participants provided written informed consent.

NucliSens and MONITOR assays were performed according to the manufacturers' instructions using 0.5 to 1.0 ml of CSF (most assays used 1.0 ml). Statistical analyses were performed using SPSS version 9.0 (SPSS, Chicago, Ill.). For each subject, the mean of 17 samples obtained over 48 h at baseline (pretreatment) was used to calculate variance. All analyses used $\log_{10}$-transformed results.

For all CSF samples, HIV-1 RNA concentration ranged from 65 to 588,844 copies/ml. Viral RNA was detected in every sample by both assays. Results were less than 1,000 copies/ml for 21 (14.9%) of the NucliSens assays and 7 (5.0%) of the MONITOR assays. Results by the two methods were highly correlated (Fig. 1A) ($r = 0.826$; $P < 0.001$). The mean HIV-1 RNA result by the MONITOR assay was 4.01 $\log_{10}$ copies/ml, and that by the NucliSens assay was 3.71 $\log_{10}$ copies/ml. For individual samples, MONITOR assay results were 0.29 ± 0.4 $\log_{10}$ copies/ml (mean $\pm$ standard deviation) greater than NucliSens assay results. This difference was apparent at both high and low HIV-1 RNA concentrations (Fig. 1B). However, for 29 (20.6%) of the paired assays, the NucliSens result exceeded the MONITOR result. MONITOR results exceeded NucliSens results by at least 1.0 $\log_{10}$ in 5 (3.5%) of samples. For samples with NucliSens assay results between 3.5 and 4.5 $\log_{10}$ copies/ml, the difference between assays was somewhat less. Within this range, MONITOR assay results were only 0.13 ± 0.34 $\log_{10}$ copies/ml greater than NucliSens assay results.

We next examined assay variance about the mean for each subject at baseline (Table 1). We previously established that baseline HIV-1 RNA levels in CSF were constant over 48-h intervals in these patients (11). Mean variance did not differ significantly between MONITOR (0.036 $\log_{10}$ copies/ml) and NucliSens (0.019 $\log_{10}$ copies/ml) assays ($P = 0.20$).

TABLE 2. Effect on accuracy of performing replicate HIV-1 RNA assays with CSF

$\log_{10}$copies/ml	% of means within range of actual value when the indicated no. of assays was used to calculate each mean				
	1	2	3	4	5
NucliSens					
$\pm$ 0.1	60.3	73.7	84.9	91.8	95.3
$\pm$ 0.2	91.2	97.4	99.2	99.8	100.0
$\pm$ 0.3	97.1	99.8	100.0	100.0	100.0
MONITOR					
$\pm$ 0.1	39.7	56.1	68.8	77.2	83.4
$\pm$ 0.2	69.1	87.3	94.0	97.2	98.8
$\pm$ 0.3	91.2	96.9	99.2	99.9	100.0

Inherent assay variability for plasma HIV-1 RNA level determinations is approximately 0.3 $\log_{10}$ copies/ml. While this is acceptable for clinical practice, more precise quantitation may be necessary for research studies of HIV-1 pathogenesis and treatment effect in CSF, especially if only single-timepoint CSF samples are available for analysis. We therefore characterized the extent to which repeated assays on baseline samples improved accuracy. The mean of 17 baseline CSF HIV-1 RNA determinations was assumed to represent the actual baseline HIV-1 RNA concentration. In addition to single determinations, for each patient we calculated means for every possible combination of two, three, four, or five baseline assays (Table 2). For 17 separate baseline assays there are 6,188 unique five-way combinations from which means can be calculated.

Single assays for both the NucliSens and MONITOR methods provided results within 0.3 $\log_{10}$ copies/ml of the actual value in more than 90% of cases. By comparison, to achieve results within 0.2 $\log_{10}$ copies/ml of the actual value in more than 90% of cases required single NucliSens assays and triplicate MONITOR assays. To achieve results within 0.1 $\log_{10}$ copies/ml of the actual value in more than 90% of cases required four NucliSens assays and more than five MONITOR assays. These results suggest that the NucliSens assay can achieve somewhat greater accuracy with fewer replicates.

In summary, this study of HIV-1 in CSF established a strong correlation between results generated by NucliSens and MONITOR assays. MONITOR assay results using CSF are on average approximately twofold higher than by NucliSens assay. This is consistent with what has been reported for plasma samples (8). For clinical practice, NucliSens and MONITOR assays are of comparable utility for quantifying HIV-1 RNA in CSF. For research studies, the NucliSens assay may achieve somewhat greater accuracy with fewer replicates.

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